

Characterization studies of novel human anti-CEA immunotoxins containing rGel

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Abstract

Carcinoembryonic antigen (CEA) is known to be widely expressed in various tumors and related with metastases so that it may be the highly potential target for therapeutically treating various tumors including colorectal, gastric, pancreatic, and breast carcinomas.

We generated novel fusion constructs composed of a single-chain anti-CEA antibody (scFvCEA) and recombinant plant toxin gelonin (rGel), which is a single-chain *N*-glycosidase similar in its action to ricin A chain as a therapeutic molecule. We introduced the leucine-rich extra amino acids at C-terminus of rGel to make a second version of fusion construct (scFvCEA-rGel-29K) enhancing dimerization in order to investigate its effect on biological activities.

Genes encoding the fusion constructs were inserted into the protein expression vector, pET32a(+), and transformed into *E. coli* strain AD494 (DE3) pLysS. The fusion proteins were expressed in the form of hexa histidine (His)-tagged proteins and purified using immobilized metal affinity chromatography. The His-tag was removed from the final product by cleavage with enterokinase (EK).

The scFvCEA-rGel targets to the CEA expressing colorectal carcinoma, pancreatic and breast tumor cell lines such as LS174T, HT29, Capan-1, and MCF-7 respectively. The scFvCEA-rGel-29K showed higher cytotoxicity than scFvCEA-rGel, which might be resulted from the dimerization and the effective localization to CEA expressing tumors.

This suggests that the fusion construct targeting CEA antigen has significant therapeutic potential for the treatment of disease states and may represent a potentially novel class of therapeutic agents. Research conducted, in part, by the Clayton Foundation for Research.

Background

We generated a fusion construct composed of a single-chain anti-CEA antibody (scFvCEA) fused to recombinant plant toxin gelonin (rGel). Secondly, we extended the C-terminus of rGel with leucine-rich extra amino acids (5'-AALEA LAEAL EALAE ALEAL AAAAA AGGCK DEL-3'), which is scFvCEA-rGel-29K. As a control, scFvCEA was expressed by the same expression system and was used to compare the biological properties with scFvCEA-rGel and scFvCEA-rGel-29K proteins.

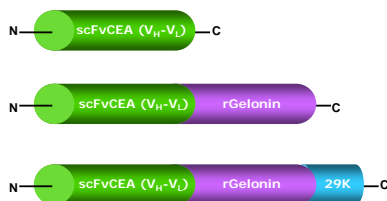
The rGel is an *N*-glycosidase cleaving adenine from mammalian ribosomal RNA and resulting in cell death. It is known that a single molecule of rGel delivered to the cytoplasmic compartment is sufficient to irreversibly intoxicate a target cell.

With targeting moiety, scFvCEA, binding to CEA expressed by the tumor cells, rGel or rGel-29K expects to efficiently internalize into the cell specifically.

Results

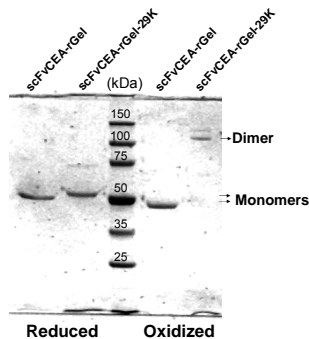
Expression Vector and Host Strain

pET32a(+)/ *Escherichia coli* AD494 (DE3) pLysS



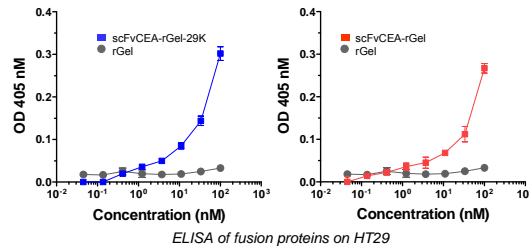
Constructions of scFvCEA (25.7 kDa), scFvCEA-rGel (54.9 kDa), and scFvCEA-rGel-29K (57.4 kDa)

Expression and Purification of Fusion Proteins

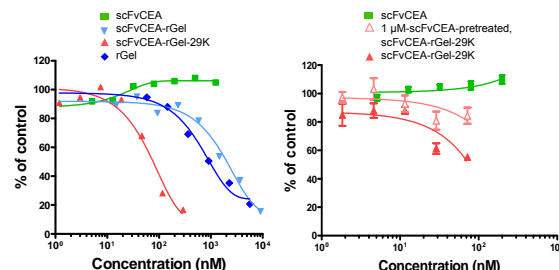


scFvCEA-rGel-29K forms dimer (114.8 kDa) whereas scFvCEA-rGel exists in monomer (54.9 kDa)

Biological Characterization of Fusion Proteins



ELISA of fusion proteins on HT29



In vitro cytotoxicity against MCF-7

Relative levels of CEA expressed intra- and extra-cellularly in tumor cells

Cell line	Cell type	[CEA] ⁺ in cell	[CEA] ⁺ in medium
LS174T	Colorectal	+++	+++
HT29	Colorectal	++	+
WDr	Colorectal	++	-
Capan-1	Pancreatic	++	-
MCF-7	Breast	+++	-

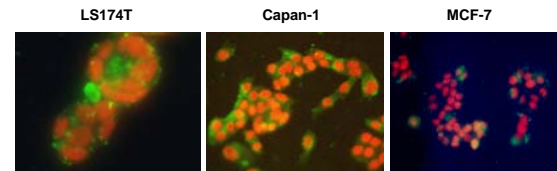
[CEA]⁺: relative expression levels compared by Western blotting using rabbit anti-CEA antibody

The IC₅₀ values of rGel, scFvCEA-rGel, and scFvCEA-rGel-29K against colorectal, pancreatic and breast cancer cell lines

Cell line	IC ₅₀			Targeting Index*	
	rGel (μM)	scFvCEA-rGel (μM)	scFvCEA-rGel-29K (μM)	scFvCEA-rGel	scFvCEA-rGel-29K
LS174T	1.7	2.6	0.3	0.7	6
HT29	7.0	9.0	0.2	0.8	35
WDr	4.0	3.4	0.2	1.2	20
Capan-1	1.1	2.0	0.2	0.6	6
MCF-7	3.4	1.8	0.2	1.9	17

* Targeting index represents IC₅₀ of rGel / IC₅₀ of fusion protein

Internalization of Fusion Protein into the Tumor Cells



Internalization of scFvCEA-rGel-29K into colorectal (LS174T), pancreatic (Capan-1) and breast (MCF-7) carcinomas assessed by immunofluorescence microscopy. Internalized scFvCEA-rGel-29K (green) into the cells were specifically detected by FITC-coupled anti-rabbit IgG treated with 400nM scFvCEA-rGel-29K for 4h at 37°C. Nuclei, shown in red, were stained as red by treatment with 1 μg/mL of propidium iodide (PI).

Conclusions

- We have generated novel fusion constructs of the human anti-CEA single-chain Fv antibody (scFvCEA) and a plant immunotoxin gelonin (rGel).
- The scFvCEA-rGel-29K forms a dimer and showed higher cytotoxicity than scFvCEA-rGel against colorectal, pancreatic, and breast cancer cell lines.
- Pretreatment of scFvCEA suppressed the cytotoxic effect, which means the cytotoxicity is mediated by binding of scFvCEA to CEA on tumor cells.
- The fusion construct was effectively internalized into LS174T, Capan-1, and MCF-7 cells.
- The fusion constructs of scFvCEA-rGel and scFvCEA-rGel-29K may have significant potential to treat tumors expressing CEA.